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## CELL GROWTH REGULATORY GENES

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### BACKGROUND OF THE INVENTION

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Significant advances have been made recently towards the identification of numerous potential growth effectors within cells. Although previous analyses have evaluated the phenotypic effects of cellular environment, growth factors, and stress, it is only recently that the underlying molecular events responsible for perturbing cell growth are beginning to be defined. In a large number of cases, the cause of a particular cellular growth response can be attributed to transcriptional attenuation. Indeed, the recent gene discoveries directly relevant to cell-cycle regulation have been based, to a significant degree, on differential gene expression analyses.

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The *p53* gene remains the most frequently mutated gene in human cancers undoubtedly reflecting an important regulatory function for this gene in controlling cell growth. *p53* function can be attenuated by interaction with viral or cellular proteins and cytoplasmic sequestration, ultimately leading to alterations in cell-growth potential. Although *p53* is thought to exert growth-regulatory functions in response to DNA damage and by directly inhibiting DNA replication, or inducing apoptosis, a large body of data indicate that the primary function of *p53* is to transcriptionally regulate downstream effector genes. *p53* contains a potent transcriptional activation domain and is able to bind DNA in a sequence-specific manner, allowing for both transcriptional activation and repression of target genes.

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Identification of the *p53* transcriptionally-responsive genes *p21<sup>WAF1/CIP1</sup>*, *MDM2*, *GADD45*, *HIC*, *cyclin G*, and *BAX*, the products of which have been

5 suggested to have direct effects on control of cell growth and/or survival, emphasizes the pivotal role of *p53* in modulating expression levels of growth-response genes. To date, two of the best characterized, direct effectors of cell growth which are transcriptionally-regulated by *p53* are *p21<sup>WAF1/CIP1</sup>* and  
10 *MDM2*. *p21<sup>WAF1/CIP1</sup>* functions as a cyclin-dependent kinase (CDK) inhibitor by directly interacting with CDK proteins during progression of the cell cycle. Overexpression of *p21<sup>WAF1/CIP1</sup>* via transfection suppresses cell growth in a wide array of cancer cell lines. In contrast, *MDM2* functions by negatively regulating *p53* activity and overexpression of *MDM2* leads to uncontrolled cell growth and tumorigenesis. However, *p53*-induced transcriptional responses appear  
15 ill-conserved, suggesting cell-type-, genetic-, and species-dependent factors may contribute to *p53*-responsiveness. For example, both *p21<sup>WAF1/CIP1</sup>* and *MDM2* are transcriptionally activated in a *p53*-dependent manner in rodent cells harboring a temperature-sensitive *p53* allele, but only *p21<sup>WAF1/CIP1</sup>* is induced in an analogous human system. In addition, activation of *p21<sup>WAF1/CIP1</sup>* has been shown to be both  
20 *p53*-dependent and independent, suggesting numerous pathways for regulation of *p21<sup>WAF1/CIP1</sup>*. Furthermore, it appears that the level of apoptotic regulatory gene products such as *BCL2* or *E1B* also aid in determining if a cell will undergo *p53*-dependent growth arrest or apoptosis. These results suggest that a complex regulatory cascade, utilizing numerous effector gene products, determines a cell's fate with regard to cell growth potential. The complex nature of cell-growth  
25 regulation underscores the need to further define the molecular components affecting cellular growth regulation.

#### SUMMARY OF THE INVENTION

It is an object of the invention to provide DNA molecules that encode  
30 mammalian cell growth regulatory proteins.

It is another object of the invention to provide mammalian cell growth regulatory proteins.

5 It is still another object of the invention to provide antibodies which selectively bind to and can be used to assay for mammalian cell growth regulatory proteins.

It is yet another object of the invention to provide methods of suppressing growth of tumor cells.

10 It is another object of the invention to provide diagnostics and diagnostic methods for cancers.

It is an object of the invention to provide an antisense construct useful for stimulating cell growth.

15 It is an object of the invention to provide antisense oligonucleotides for inhibiting expression of cell growth regulatory genes.

It is another object of the invention to provide methods for promoting proliferation of cells.

It is still another object of the invention to provide a method for assessing susceptibility to cancers.

20 These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention an isolated and purified subchromosomal DNA molecule is provided which encodes a mammalian CGR11 or CGR19 protein.

25 According to another embodiment of the invention, an isolated and purified mammalian CGR11 or CGR19 protein is provided.

In another embodiment of the invention an antibody is provided which specifically binds to a mammalian CGR11 or CGR19 protein.

According to another embodiment of the invention a method of suppressing growth of tumor cells is provided. The method comprises the step of:

30 administering to tumor cells a mammalian CGR11 or CGR19 protein.

According to another embodiment of the invention a method of suppressing growth of tumor cells is provided. The method comprises the step of:

5 administering to tumor cells a DNA molecule which causes said cells to express a mammalian protein selected from the group consisting of CGR11, CGR19, mEH, and SM20.

According to still another embodiment of the invention a method for diagnosing cancer is provided. The method comprises the steps of:

10 testing a tissue to determine if the tissue expresses less of a mammalian protein selected from the group consisting of CGR11, CGR19, mEH, and SM20 or less of an mRNA encoding the mammalian protein than a normal tissue.

15 According to still another aspect of the invention, a method for diagnosing cancer is provided. The method comprises the steps of:

testing a tissue to determine if DNA in said tissue contains a mutant form of a mammalian gene coding sequence selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*, which mutant form differs from the wild-type form of the gene coding sequence.

20 In another embodiment of the invention an antisense *CGR11*, *CCGCR19*, *mEH*, or *SM20* construct is provided. The construct comprises:

- a. a transcriptional promoter;
- b. a transcriptional terminator;
- c. a DNA segment comprising one or more segments of a gene coding sequence for a mammalian protein selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*, said gene coding sequence segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of mRNA produced by mammalian cells.

30 In another aspect of the invention, a *CGR11*, *mEH*, *SM20*, or *CGR19* antisense oligonucleotide is provided. The oligonucleotide comprises at least ten nucleotides complementary to a mammalian *CGR11* or *CGR19* mRNA.

5 According to one embodiment of the invention a method for promoting the proliferation of cells is provided. The method comprises the step of:  
10 administering an antisense oligonucleotide for a mammalian gene selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*, comprising at least ten nucleotides complementary to a mammalian gene mRNA selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20* to said cells to inhibit the expression of *CGR11*, *CGR19*, *mEH*, or *SM20*.

15 According to another aspect of the invention, a method for promoting the proliferation of cells is provided. The method comprises the step of:

20 administering a triplex-forming oligonucleotide comprising at least ten nucleotides complementary to a mammalian gene selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*, to mammalian cells to inhibit the expression of a mammalian gene selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*.

25 According to another aspect, another method for promoting growth of cells is provided. The method comprises the step of:

30 administering to mammalian cells to inhibit the expression of a mammalian gene an antisense gene construct selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*, comprising:

a. a transcriptional promoter;

b. a transcriptional terminator;

35 c. a DNA segment comprising one or more segments of the mammalian gene coding sequence, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of RNA produced by mammalian cells.

According to another embodiment of the invention, a method for assessing susceptibility to cancers is provided. The method comprises the step of:

5 testing a tissue selected from the group consisting of blood, chorionic villi, amniotic fluid, and a blastomere of a preimplantation embryo, to determine if DNA in said tissue contains a mutant gene coding sequence selected from the group consisting of *CGR11*, *CGR19*, *mEH*, and *SM20*.

10 The present invention thus provides the field with additional diagnostic and therapeutic tools with which to manage cancer risk assessment, incipient cancer, and frank cancers.

#### BRIEF DESCRIPTION OF THE DRAWINGS

15 **Figure 1.** Characterization of REF-112 RNA. Fig. 1a, Kinetics of WAF1 induction. Total RNA from REF-112 cells maintained at 32°C for the indicated times (hours) was probed with rat WAF1 cDNA. Fig. 1b, cyclin G RT-PCR. Total RNA harvested from REF-112 cells maintained at 38°C, or 32°C for 8 hours, was reverse-transcribed with an anchored oligo-dT primer. PCR was performed with this primer and a primer specific for the 3' end of rat cyclin G. The arrowhead shows an ≈300 base pair, differentially-expressed rat cyclin G RT-PCR product.

20 Fig. 1c, Northern analysis of total RNA from REF-112 cells. The cyclin G RT-PCR cDNA described in Fig. 1b was used as a probe for RNA isolated from 38°C or 32°C maintained REF-112 cells.

25 **Figure 2.** Differentially expressed genes from wild-type *p53*-containing REF-112 cells. RT-PCR and Northern analysis from 38°C and 32°C (8 hours) REF-112 cells representing *CGR11* (Fig. 2a), *CGR19* (Fig. 2b), *SM20* (Fig. 2c), and *mEH* (Fig. 2d). Arrows indicate differentially-expressed cDNA which was excised, reamplified, and used as a probe for the accompanying Northern analysis. Lane 1, Lane 3; 32°C-maintained REF-112 RNA harvested after an 8 hour incubation. Lane 2, Lane 4; 38°C REF-112 RNA. Duplicate samples are independently isolated total RNA preparations.

30 **Figure 3.** Expression profiles for *CGR11* and *CGR19*. Fig. 3a; Rat multiple tissue Northern blot probed with <sup>32</sup>P-labeled cDNA fragments specific for *CGR11*, *CGR19*, and β-actin. Fig. 3b, Induction kinetics of *CGR11* and *CGR19*. REF-112 cells were maintained at 32°C for the indicated times (hours). Total RNA

5 was isolated, electrophoresed, blotted, and probed with  $^{32}\text{P}$ -labeled cDNA specific for rat *CGR11* or *CGR19*.

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Figure 4. Amino acid sequence of rat and human *CGR11* and *CGR19*. Fig. 4a; Dduced amino acid sequence of human (top line) and rat (bottom line) *CGR11*. Dashes represent identity between the human and rat sequences. Asterisk represent gaps present within the rat *CGR11* sequence. The overline represents the two putative EF-hand domains. Fig. 4b, *CGR11* EF-hand domains I and II aligned with the consensus EF-hand sequence. Rat amino acids differing from the human sequence are shown on the bottom line. Fig. 4c, Dduced amino acid sequence of human (top) and rat (bottom) *CGR19*. The overlined sequence encodes a potential zinc-binding, ring-finger domain. Dashes indicate identity between the human and rat sequences. Fig. 4d, *CGR19* ring-finger domain aligned with the consensus ring-finger sequence.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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The present inventors have discovered that four genes not previously known to be regulated by *p53* are regulated by *p53* and are shown herein to be growth-regulatory. Two of the genes isolated, rat *SM20* and rat and human *mEH* are previously described genes, although no correlation between *p53* status and transcriptional induction of these genes has been previously established. The other two genes, *CGR11* and *CGR19*, have not been isolated or described previously.

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Rat *SM20* was first isolated via an analysis of *PDGF-A*-induced transcripts in vascular smooth muscle cells, however, the function of rat *SM20* has not yet been elucidated. In contrast, *mEH* is known to be involved in the catalytic detoxification of xenobiotics, including metabolizing reactive epoxides. While no known correlation exists between *mEH* and wild-type *p53*, induction of *p53* activity through epoxides may aid in maintaining genomic stability in the presence of toxins.

We utilized the well-characterized murine temperature-sensitive *p53* mutation VAL135 to identify transcriptional responses of wild-type *p53* protein. Growth of rat embryo fibroblast cells (REF-112) transformed with activated RAS and *p53*-VAL135 is temperature sensitive. Growth of these cells at 38°C maintains the

5 p53 protein in a mutant conformation complexed with HSP70 in the cytoplasm. When REF-112 cells are shifted to 32°C, the p53 protein adopts the wild-type conformation, migrates into the nucleus and regulates transcription of target genes. These cells were initially described as growth-arrested predominantly in the G1 phase of the cell cycle. We employed a differential transcript analysis from rat cells 10 temperature-sensitive for p53 function. We identified those transcripts that were present at the permissive temperature, but not present at the non-permissive temperature.

DNA molecules according to the present invention are isolated and purified 15 from other gene sequences. They may be either genomic sequences or cDNA sequences, *i.e.*, they may or may not contain intervening sequences. The nucleotide sequence of the coding region of human CGR11, human CGR19, rat CGR11, and rat CGR19 are shown in SEQ ID NOS: 1, 3, 5, and 7, respectively. Other mammalian homologues can be readily obtained by screening cDNA libraries using 20 nucleotide probes or primers derived from the disclosed sequences. Genomic clones can also be obtained by screening genomic DNA libraries, such as YAC or P1 clones using probes derived from the gene coding regions. Allelic forms of the genes are also encompassed by the present invention. These may have either silent mutations which do not affect the coding sequence, or polymorphisms which lead 25 to amino acid differences. The amino acid differences may or may not cause a change of protein function.

Using the protein sequences shown in SEQ ID NOS: 2, 4, 6, and 8, one can 30 also identify and obtain the corresponding genes and related mammalian CGR genes using degenerate oligonucleotide probes. One or more degenerate probes can be used to hybridize to cDNA or genomic DNA libraries. CGR genes can be identified by hybridization to the degenerate probes. The identity of the genes can be confirmed on the basis of one or more of the following properties: the approximate size of the encoded protein, the similarity or identity to the proteins of SEQ ID NOS: 2, 4, 6, or 8, and the upregulation of the mRNA for the gene by wild-type p53. Design of and conditions for use of degenerate probes are well known in the

5 art. Portions of the full length gene or cDNA can be isolated similarly, with or without isolating the full-length gene. Alternatively, antibodies which specifically bind to CGR protein(s) can be raised against proteins or polypeptide portions of the proteins shown in SEQ ID NOS: 2, 4, 6, and 8. These antibodies can be used to select clones in an expression cDNA library which express the same or related epitopes. Portions of CGR genes which are identified can be made by fragmentation of the gene by restriction endonucleases or by amplification of a partial sequence. Portions of CGR genes which are isolated by hybridization or by antibody binding can also be used directly, without isolating the whole gene.

10 Now provided with the sequences of the four mammalian cell growth regulatory genes, one of ordinary skill in the art can readily obtain the encoded proteins. They can be expressed in bacteria, yeast, or other convenient cell. Expression vectors can be constructed by ligation of the coding regions into vectors which are known in the art. Typically the vector will contain expression control sequences, such as promoters, enhancers, termination of transcription signals, etc. 15 Thus by placing the coding sequence in the correct position and orientation with respect to the expression control sequences, an expression vector is obtained which is capable of directing the expression of the selected mammalian protein in a desired host cell. Suitable vectors are known in the art and can be based on plasmids or viral genomes. Appropriate host cells for vectors are also known, and can be selected for the desired properties which they display.

20 25 Portions of the selected protein can be synthesized and linked to a carrier protein for immunization of laboratory animals to raise antibodies specifically immunoreactive with them. The antibodies can be used to purify the proteins from natural or recombinant sources. Such antibodies can be polyclonal or monoclonal, as is convenient for the particular application. Antibodies which bind specifically to the disclosed CGR proteins can be readily isolated using routine screening, as is well known in the art.

30 As described herein, cell growth regulatory proteins have a growth-suppressing effect on tumor cells. Thus, their administration to tumor cells, or the

5 administration of their corresponding genes in a construct that will result in expression of the proteins, may be desirable to effect such growth suppression. The proteins of the present invention may be formulated as pharmaceutical compositions, for administration to humans. Typically these will be sterile formulations in a diluent or vehicle which is free of pyrogenic components. The formulations are suitable for either intranasal and parenteral administration. Other cells which are involved in proliferative diseases may also be targeted for cell growth regulatory gene-mediated growth suppression. Such proliferative diseases include and are not limited to psoriasis, polyps, restenosis, warts, and inflammatory diseases. Cell 10 growth regulatory proteins may be administered in suitable formulations to tumor cells. They may be microinjected, or simply supplied externally to tumor cells. They may be encapsulated, e.g., in liposomes. If administered to a mammal, it is desirable to achieve a tumor suppressing amount in the circulation. A tumor-suppressing effect can be achieved if a concentration of  $10^{-10}$  to  $10^{-6}$  M of the cell 15 growth regulatory protein is achieved. If cell growth regulatory protein-encoding DNA is administered to tumor cells then the cells can express their own cell growth regulatory protein for growth suppression. Such DNA can be genomic or cDNA, as described above. Other cells involved in proliferative diseases may be treated 20 similarly.

25 The cell growth regulatory genes of the present invention are shown here to be regulated by wild-type p53. Therefore, one can use the expression of any of the cell growth regulatory genes as a marker for the expression of wild-type p53. Diminished cell growth regulatory gene expression, relative to normal tissues, can indicate cancer, just as diminished wild-type p53 expression or presence of mutated p53 expression can be indicative of cancer. Assays for cell growth regulatory gene 30 expression can be used in addition to, or in place of, assays for wild-type p53 directly. Tissues which are suitable for comparison purposes to provide a normal control are typically adjacent, morphologically normal tissues. Tests for the presence or amount of cell growth regulatory gene expression can employ either antibodies specific for a cell growth regulatory protein, nucleic acid probes or

5 primers of at least about 10 nucleotides complementary to all or a portion of the sequence of SEQ ID NOS: 1, 3, 5, or 7, or other tests known in the art.

10 Similarly, DNA of a tumor tissue can be tested to determine whether it contains mutations. Cell growth regulatory gene mutations confer a neoplastic phenotype on cells, as do p53 mutations. Mutations can be determined by determining the sequence of the genes in the tissue being tested, and comparing that sequence to those disclosed in SEQ ID NOS: 1, 3, 5, or 7. Such mutations may arise in the germline or in somatic tissues. If the mutations arise in somatic tissues, then they will not be found in other tissues of the same individual. If the mutations arise in the germline, they will be found in all tissues of the body, and will, like germline p53 mutations, indicate a susceptibility to cancers. Tissues suitable for testing for germline mutations include blood, mucosal smears, cervical smear, skin, chorionic villi, amniotic fluid, and blastomeres of preimplantation fertilized embryos.

15

20 Antisense cell growth regulatory gene constructs contain a transcriptional promoter and a transcriptional terminator (polyadenylation signal), with a DNA segment between them. The DNA segment comprises one or more segments of the cell growth regulatory gene, but that segment(s) is in an inverted orientation in the construct, compared to the orientation in the mammalian genome. Transcription from the transcriptional promoter of the construct produces an (antisense) RNA molecule which is complementary to cell growth regulatory gene RNA which is produced from the cell growth regulatory promoter in normal mammalian cells. The promoter used to make the antisense RNA molecule can be an inducible promoter which can be regulated by certain prescribed stimuli. For example, a metallothionein promoter or a hormone responsive promoter can be advantageously used. Other promoters and terminators can be used as is convenient in the particular application. In addition, enhancers known in the art can be used to enhance expression of the desired proteins.

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30

The antisense cell growth regulatory gene constructs of the present invention can be used in one type of cell to produce antisense RNA which is then applied to

5 other cells by techniques known in the art. Alternatively, the cell growth regulatory gene constructs can be administered to the ultimate target cells in which regulation of a cell growth regulatory gene is desired. Suitable means for introducing DNA constructs into cells are known in the art. Administration of antisense constructs may be by transfection, transformation, electroporation, fusion, etc., as is known in the art. Inhibition of cell growth regulatory gene expression causes cells to proliferate and prevents cell death. This can be particularly useful in situations where growing large numbers of certain cells in culture is desirable, such as in the case of culturing epidermal cells for transplantation. Alternatively, administration to certain cells of the body may be desirable, such as to aging or senescent cells to prevent senescence, or to immune cells or cells of the gastrointestinal tract.

10 Cell growth regulatory gene antisense oligonucleotides are also provided for the same purpose as the antisense constructs, discussed above. The oligonucleotides are at least ten nucleotides and may be twenty or thirty nucleotides in length. They may consist of normal nucleotides or nucleotide analogues or mixtures of the two.

15 Analogues include methylphosphonates, aminoalkylphosphonates, phosphorothioates, phosphorodithioates, substituted or unsubstituted phosphoramidates. The antisense oligonucleotides are typically linear, single-stranded molecules which are complementary to the natural cell growth regulatory gene mRNA made by mammalian cells, though circular molecules can also be utilized. These can be administered to cells in liposomes, or naked, for uptake by the cells by passive or receptor-mediated transport. It is often desirable that the antisense oligonucleotide be designed to be complementary to the 5' end of the mRNA, in particular to the translation start site. However, other portions of mRNA molecules have been found to be amenable to antisense inhibition, and may be used in the practice of the present invention. It is also desirable to avoid portions of the mRNA as target for the antisense oligonucleotides which have secondary structures which involve hydrogen bonding with other portions of the molecule. For example, it is desirable to avoid regions which appear to be involved in formation of stems of stem-loop structures.

5           The expression of a cell growth regulatory gene may also be inhibited by interference with transcription, by adding oligonucleotides or modified oligonucleotides that can form triple-stranded structures (triplexes) by complexing with a segment of the cell growth regulatory gene.

10          The following examples are provided for exemplification purposes only and are not intended to limit the scope of the invention which has been described in broad terms above.

### EXAMPLES

#### Example 1

15          Pilot studies demonstrate the usefulness of the temperature-sensitive p53 system.

20          The feasibility of differential display analysis utilizing REF-112 cells was evaluated by first characterizing total RNA prepared from cells grown at both 38°C and 32°C for known p53 regulated genes. Northern analysis of *p21<sup>WAF1/CIP1</sup>* showed a rapid induction of transcription, with *p21<sup>WAF1/CIP1</sup>* RNA levels peaking 8-10 hours after shifting to 32°C (Fig. 1a). Furthermore, we have demonstrated similar kinetics of induction of apoptosis within 8-10 hours after shifting to 32°C.

25          We chose an eight hour induction time for the differential transcript analysis presented here. At this time rat *p21<sup>WAF1/CIP1</sup>* and rat *cyclin G* northern analysis showed high levels of each transcript at the growth-inhibitory temperature of 32°C and little or no transcript in cells growing exponentially at 38°C (Fig. 1). Both *cyclin G* and *p21<sup>WAF1/CIP1</sup>* appeared to be induced to similar levels.

30          RT-PCR reactions were performed on total RNA isolated from both growth conditions with primers specific for the 3' end of rat *cyclin G* to characterize the RNA preparation prior to identifying novel p53-regulated genes. As anticipated, a differentially-expressed band of 300 bp was detected in the 32°C-induced RNA, but was absent in the RNA from uninduced cells (Fig. 1b). Excision of the *cyclin G* RT-PCR band and subsequent cloning and Northern analysis (Fig. 1c) confirmed the identity of this band as rat *cyclin G* (data not shown).

5           *Cell culture:* Rat embryo fibroblast cells REF-112 (p53 -VAL135) and  
REF-132 (p53 -PHE132) (kindly provided by B. Vogelstein and M. Oren), were  
grown in DMEM containing 10% fetal bovine serum in 5% CO<sub>2</sub> at either 38°C or  
32°C. Cells were split and seeded 48 hours before any temperature shifts.  
Temperature shifts were performed by simple transfer of subconfluent flasks to  
10 pre-equilibrated incubators without media changes. For transfections, 4 x 10<sup>5</sup> cells  
(T98G and SW480 cells), or 0.8 x 10<sup>5</sup> cells (SKOV3-IP1) were seeded in 6-well  
dishes. Transfections were performed using lipofectin (Gibco/BRL) as described  
by the manufacturer. Briefly, 40 µl of reduced serum medium (OPTIMEM™,  
Gibco/BRL) was added to 2 µg DNA. A 50 µl mixture containing 10 µl lipofectin  
15 and 40 µl OPTIMEM™ was added to the DNA mixture. After a 15 minute room  
temperature incubation, 1 ml of OPTIMEM™ was added and the mixture was  
overlaid onto optimem-washed cells. Cells were allowed to incubate for five  
hours in a 37°C/5% CO<sub>2</sub> incubator following which the transfection mixture was  
replaced with normal growth medium. After 44 hours, cells were split into  
20 selection medium containing hygromycin (0.25mg/ml). After 12-14 days, colonies  
were stained with 2% methylene blue in 50% ethanol and counted. Only colonies  
containing > 50 cells were scored.

25           *RNA Isolation:* Total RNA was isolated by direct lysis in RNazol B  
(Tel-Test, Inc.) as described by the manufacturer. Poly A<sup>+</sup> RNA was isolated from  
total RNA preparations using a MessageMaker kit (Gibco/BRL) as described by the  
manufacturer.

30           *RT-PCR Reactions:* Reverse transcription reactions were performed using  
200 ng total RNA in 5 mM MgCl<sub>2</sub>/10 mM Tris, pH 8.3/10 mM KCl/20 µM  
dNTP's/20 units RNase inhibitor (Perkin Elmer)/50 µM T<sub>12</sub>NN and 50 units  
MMLV reverse transcriptase (Perkin Elmer) by heating samples (without reverse  
transcriptase) to 65°C for 5 minutes, and then placing the reaction at 37°C for 5  
minutes. Reverse transcriptions were allowed to proceed for 55 minutes at 37°C.  
RT reactions were inactivated by incubating for 5 minutes at 95°C. PCR reactions  
were performed with 2 µl of the reverse transcriptase reaction in 1.5 mM MgCl<sub>2</sub>/10

5 mM Tris pH 8.3/10 mM KCl/4  $\mu$ M dNTP's/2.5 units Taq polymerase (Perkin  
Elmer)/10  $\mu$ Ci  $^{35}$ S- $\alpha$ -dATP (12.5  $\mu$ Ci/ $\mu$ l, NEN)/50  $\mu$ M T<sub>12</sub>NN and 0.2  $\mu$ M random  
10-mer. PCR reactions were performed in a thermocycler for 40 cycles of: 94°C  
for 3 seconds/40°C for 2 minutes/72°C for 30 seconds. Reactions were terminated  
by the addition of 38% formamide/8 mM EDTA/0.02% bromophenol blue and  
10  
xylene cyanol. Samples were heated for 2 minutes at 70°C and run on a 6%  
denaturing polyacrylamide gel. PCR products were purified after drying the gel  
onto Whatmann 3MM paper and exposure to film. Excised bands were resuspended  
15 in 120  $\mu$ l H<sub>2</sub>O for 10 minutes at room temperature, followed by 15 minutes of  
boiling. Debris was pelleted by centrifugation and 10  $\mu$ l 3M sodium acetate, 5  $\mu$ l  
10 mg/ml glycogen, and 400  $\mu$ l ethanol was added to 100  $\mu$ l of the eluted DNA.  
DNA was allowed to precipitate overnight at -20°C. The DNA was pelleted,  
amplified using the same primers used for the original RT-PCR, gel purified, and  
cloned into pCRII (InVitrogen).

20 *Oligonucleotides:* For cyclin G RT-PCR reactions, dT<sub>12</sub>GC and  
5'-TCTTCACTGC-3' primer pairs were used to amplify a ~300 bp fragment.

25 *Northern analysis:* Northern analysis was performed using 10-20  $\mu$ g total  
RNA electrophoresed on 1.2% formaldehyde gels. Blotting and probing was  
essentially as described. Probes were gel-purified cDNA fragments  $\alpha$ -<sup>32</sup>P-labeled  
by random priming (BMB). Rat tissue blots were obtained from Clonetech and  
probed, stripped, and reprobed as recommended by the manufacturer. Rat WAF1  
cDNA was kindly provided by B. Vogelstein.

#### Example 2

This example demonstrates the use of the temperature-sensitive p53 system  
to identify p53 regulated genes.

30 To identify other growth-regulated genes we performed RT-PCR reactions  
utilizing 12 "anchored" oligo-dT primers in conjunction with 25 "random" 10-mers.  
RT-PCR reactions were performed on duplicate, independently-isolated total RNA  
from REF-112 cells maintained at 38°C or 32°C. Primer-pairs that would amplify  
either rat p21<sup>WAF1/CIP1</sup> or MDM2 were omitted.

5           A total of 35 differentially-expressed RT-PCR products were chosen for further analysis based on induction of product solely at the induced temperature of 32°C. Although RT-PCR reactions were performed on duplicate RNA samples, all reactions containing potentially interesting transcripts were repeated in full to further avoid potential artifacts, a common problem in differential display analyses .  
10           Subsequent northern analysis ruled out all but four of these RT-PCR products as being differentially-expressed genes. None of these four genes showed any transcriptional induction in RNA from control cells harboring a non-temperature-sensitive *p53* mutation (REF-132 cells [PHE132]) grown at 32°C, suggesting that the transcriptional induction observed was not due only to the shift in temperature, but also due to the mutation. Two of the induced genes have been previously described including *SM20* and microsomal epoxide hydrolase (*mEH*, ). Although correlative gene expression analyses have described these genes as being differentially expressed depending on growth conditions, transcription of these genes has not previously been linked to *p53* expression.  
15  
20

Differential RT-PCR products from the two novel cDNA's, designated 11 and 19, as well as *SM20* and *mEH* are shown in Figure 2. Northern analysis for each of these partial cDNA's confirmed that these transcripts were differentially expressed in cells grown at 38°C or 32°C (Fig. 2).

25           **Oligonucleotides:**       Twelve anchored oligo-dT primers (dT<sub>12</sub>[A,C,G][A,C,G,T] combinations) and 25 random 10 mers were used for RT-PCR reactions. For cyclin G RT-PCR reactions, dT<sub>12</sub>GC and 5'-TCTTCACTGC-3' primer pairs were used to amplify a ≈300 bp fragment. RT-PCR amplification of specific clones was achieved using the following primer pairs: *CGR11*: dT<sub>12</sub>AT/TACAACGAGG; *SM20*: dT<sub>12</sub>GG/GATCATAGCG; *CGR19*: dT<sub>12</sub>CG/GATCATAGCC; *mEH*: dT<sub>12</sub>CA/GATCATGGTC.  
30

Example 3

This example demonstrates the tissue-specific expression of the newly identified genes, *CGR11* and *CGR19*.

5           Partial cDNA fragments 11 and 19 were used as probes for expression analysis from various rat tissues (Fig. 3a). A restricted expression pattern was observed using the partial 11 cDNA probe revealing a 1.3 kb transcript present predominantly in whole brain and kidney and limited expression in heart, lung, liver, and skeletal muscle, and no detectable expression in spleen and testis. Use  
10           of the partial cDNA 19 as a probe revealed a more ubiquitous expression pattern with a 1.4 Kb transcript showing highest levels of expression in rat testis. Since neither of these genes has previously been described, we have chosen to name them Cell Growth Regulatory genes *CGR11* and *CGR19*.

15           Example 4

This example demonstrates the kinetics of expression upon temperature shift of the cell growth regulatory genes, as well as the quantitation of expression levels of the genes.

20           Kinetics of induction for *CGR11* and *CGR19* transcripts following a shift in temperature from 38°C to 32°C in REF-112 cells was performed to aid in determining whether the transcriptional induction of these genes was an early event in the cascade leading to perturbation of growth (Fig. 3b).

25           Induction kinetics closely paralleled *p21<sup>WAF1/CIP1</sup>* for *CGR19* (Fig. 3b). A slightly slower induction rate was observed for *CGR11*. This suggests that the induction of these genes is a relatively early event in the cascade leading to growth inhibition. Expression levels of the induced gene transcripts varied significantly with *CGR11* being the more highly expressed in 32°C-induced REF-112 cells (Fig. 3b). However, a higher basal (uninduced) transcript level existed for the *CGR19* than for *CGR11* (Fig. 2). Expression levels of *CGR11* and *CGR19* could also be indirectly assessed relative to *p21<sup>WAF1/CIP1</sup>* and *cyclin G* in this REF-112 system. Probe hybridization to an induced REF-112 cDNA library yielded *cyclin G* levels two fold higher than *p21<sup>WAF1/CIP1</sup>*, with *p21<sup>WAF1/CIP1</sup>* being expressed at ≈0.1% of the total mRNA in the cell and *cyclin G* being expressed at ≈0.2%.

30           Example 5

5        This example provides a sequence analysis of the isolated cell growth regulatory genes.

Potentially full-length cDNA's for *CGR11* and *CGR19* were obtained from REF-112 RNA by 5' RACE (rapid amplification of 5' ends) and by hybridization to a human, fetal brain cDNA library. Rat and human *CGR11* cDNAs obtained are 10 1,209 and 1,113 bp in length. 5' RACE analysis did not yield cDNA's with longer 5' extensions for the human clone. The rat and human cDNAs for *CGR11* have open reading frames encoding protein products of 272 and 301 amino acids, respectively (Fig. 4a). No in-frame stop codons were observed for either cDNA upstream of the putative initiation codon. Optimal 5' alignment between the rat and 15 human *CGR11* proteins truncates seven amino acids (MSRWLMQ) from the first rat *CGR11* ATG; thus a definitive designation of the rat ATG start codon can not be made. The rat and human *CGR11* proteins are 65% identical. In addition, two highly conserved putative  $\text{Ca}^{2+}$ -binding EF-hand motifs (aa 82-94 and aa 127-139 in the human protein; overlined in Figure 4b) share nearly 100% identity. Four clustered 17-amino acid repeats exist within the carboxy-terminal portion of only the 20 human *CGR11* protein (consensus: PGPRGEAEGQAEA[K/R]GDA) suggesting a structure resembling 4 alpha-helical domains interrupted by distinct turns.

25        *In vitro* production of rat *CGR11* and *CGR19* proteins yielded products of  $\approx$ 37 Kd and 34 Kd, respectively. The *CGR11* protein is very acidic with a net calculated negative charge of -29 and a pI predicted to be 4.21.

The rat and human *CGR19* proteins are 85% identical at the amino acid level 30 with consistent homology throughout the entire length of the 332 amino acid proteins (Fig. 4c). While no in-frame stop codons are found upstream from the putative initiation codon, the rat and human protein homologues diverge immediately upstream of the start codon, further suggesting that the designated ATG is indeed the start codon for *CGR19*. The predicted protein sequence of *CGR19* suggests substantial homology to known proteins only in the region of a putative zinc-binding C<sub>3</sub>H<sub>4</sub> ring-finger domain at the amino-terminus of the protein (Fig.

5        4d). No accompanying B-box domain was observed in *CGR19*, as has been  
observed in some ring-finger-containing proteins.

10      We have also isolated a full-length *CGR19* cDNA from REF-112 cells  
containing a 130 bp insert within the middle of the cDNA. This insert contain 5'  
GT and 3' AG ends consistent with exon-intron boundaries. This suggests that  
either some of the *CGR19* transcript in the cells is incompletely processed, or that  
an alternate transcript containing a retained intron is produced. This second *CGR19*  
would produce an in-frame termination codon within the putative intron producing  
a protein chimera retaining the first 140 amino acids of *CGR19*. Furthermore,  
15     RT-PCR analysis from 32°C -induced REF-112 cells reveals two bands of equal  
intensity consistent with the stable generation of two *CGR19* transcripts. Further  
analysis of potential differential protein products made from these two transcripts  
is required to determine whether this differentially-processed transcript is  
significant.

20      *5' RACE*: Rapid amplification of cDNA ends was performed with the  
marathon kit (Clonetech) using REF-112 RNA harvested from 32°C-maintained cells  
as described by the manufacturer. Oligonucleotides specific for the 3' ends of rat  
*CGR11* and *CGR19* were used in the procedure.

25      *DNA Sequencing*: Sequencing of all cDNA clones was performed by  
manual, Sanger dideoxy sequencing (USB) utilizing primers that allowed for  
sequence determination of both strands along the entire length of the cDNA's.  
Multiple clones were sequenced for both rat and human *CGR11* and *CGR19*  
cDNA's.

30      *Database searching*: All homology searches (FASTA), alignments  
(BESTFIT) and structural features (MOTIFS) were performed using The Genetics  
Computer Group (GCG) software programs (Madison, WI). Final searches were  
performed utilizing GenBank version 91.

#### Example 6

This example demonstrates the growth-suppressive properties of the cell  
growth regulatory genes.

5 To assess the potential growth-suppressive properties of the differentially-expressed genes isolated in this study, we evaluated growth inhibition via stable transfection in a colony inhibition assay. All genes examined were expressed from the cytomegalovirus (CMV) promoter on episomally-maintained plasmids (pCEP4) and colony formation was scored two to three weeks after 10 transfection. As controls, *p21<sup>WAF1/CIP1</sup>*, *p53*, and *p53* antisense constructs were also transfected. We chose three cell lines all harboring different *p53* alleles for the analysis of growth-suppressive function: The SW480 colon carcinoma cell line contains two point mutations, HIS273 and SER309; an ovarian carcinoma line, SKOV3 IP1, which is *p53*-null; and a glioblastoma cell line, T98G, containing a 15 single point mutation, MET237, in *p53*.

Rat *mEH*, *SM20*, *CGR11*, and *CGR19* all exhibited some growth-suppressive effects but to varying degrees depending on the cells analyzed (summarized in table I). The variation suggests that there is an aspect of organ specificity to the growth-suppressive effects. *p53* consistently showed the most potent growth inhibitory effect. *p21<sup>WAF1/CIP1</sup>* exhibited between 65-80% inhibition, in agreement with previous results. Only *CGR11* and *SM20* showed significant growth inhibition in SW480 cells (80% and 85%, respectively) but all clones tested showed growth inhibition in SKOVIP1 and T98G cells (70-95% inhibition) demonstrating potential conservation of *CGR11* and *CGR19* function among human cells.

25 Although the above results are suggestive of a growth-inhibitory function for the tested cDNA's, it remains possible that overexpression of these proteins yield the inhibitory effects observed due to non-specific toxicity. To address this possibility we constructed an EF-hand deletion mutant within the human *CGR11* cDNA and assessed this protein for growth-inhibitory potential. Previous results with EF-hand-containing proteins suggests that a deletion of one of two EF-hand motifs resulted in a functional protein with respect to Ca<sup>2+</sup>-binding potential. Therefore, we deleted both of the EF-hand domains within human *CGR11* for this 30 analysis (*CGR11ΔEF*, amino acids 82-139). EF-hand-deleted *CGR11* cDNA transfectants were unable to inhibit colony formation in SKOV3 IP1 cells (Table 1).

5 Human wild-type *CGR11* cDNA suppressed cell growth to 8% (SKOV3 IP1) and 58% (T98G) relative to the tested mutant. Thus it likely that, at least with respect to *CGR11*, the growth-suppressive effects observed reflects a structure-function relationship critical to growth inhibition. A similar analysis is ongoing with respect to the ring-finger domain within *CGR19*.

10 The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since they are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.

15

5

SEQUENCE LISTING

SEQ ID NO 1 human CGR11 DNA  
SEQ ID NO 2 human CGR11 protein  
SEQ ID NO 3 human CGR19 DNA  
10 SEQ ID NO 4 human CGR19 protein  
SEQ ID NO 5 rat CGR11 DNA  
SEQ ID NO 6 rat CGR11 protein  
SEQ ID NO 7 rat CGR19 DNA  
SEQ ID NO 8 rat CGR19 protein

15

## SEQ I.D. NO.: 1 &amp; 2

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-74	ggcgccgcacgagcaggagcgcacggagctggaccccaagagccgcgcgcgcga	-15
-14	gcagttccaggaaggatgttacccgtacgatgacagtgttaatccctgtgtgtcccc	45
1	M L P L T M T V L I L L L P	15
46	acgggtcaggctgcccaaaggatggagtcacaaggccagactctgaagtgcagcatcag	105
16	T G Q A A P K D G V T R P D S E V Q H Q	35
106	ctccgtcccaacccttccagccaggccaggagcactcgacttctgcagagctaccta	165
36	L L P N P F Q P C Q E Q L G I L Q S Y L	55
166	aagggacttaggaaggacagaagtgcactggagcatctgagccgggagcagggttcttc	225
56	K G L G R T E V Q L E H L S R E Q V L L	75
226	tacctcttgcctccatgactatgaccagagtcggatggatggccctggagctgtg	285
76	Y L F A L H <u>2</u> Y D O S G O I D G L E L L	95
286	tccatgttgcacagctgtctggcccctggagctgccaacttccattaccaccaaccggtg	345
96	S M L T A A L A P C A A N S P T T N P V	115
346	atattgtatgtggacaaaatgtgtcgagacgcggaccctgtaatggggatgggtcatgacc	405
116	I L I V D K V L E T Q D L N G D C L M T	135
406	cctgctgagctcatcaacttcccccggagtagccctcaggcacgtggagccggagagccc	465
136	<u>P A E L</u> I N F P G V A L R H V E P G E P	155
466	cctgctccatctctcaggagccacaagctgttggaaaggcagtccttattagctaaaagc	525
156	L A P S P Q E P Q A V G R Q S L L A K S	175
526	ccattaagacaagaaacacagggaaagccctggtcccagagaagaagcaaaggccaggta	585
176	P I R Q E T Q E A I P G P R E E A K G Q V	195
586	gaggccagaaggagtcctggatccgtccaggagcctggggccaggcagaggctgtat	645
196	E A R R E S L D P V Q E P G C G Q A E A D	215
646	ggagatgttccaggcccagaggggaagctgagggccaggcagaggctaaaggagatgcc	705
216	G D V P C P R G E A E G Q A E A K G D A	235
706	cctggcccagaggggaagctgagggccaggcagaggctaaaggagatgcccctggccc	765
236	P G P R G E A E G Q A E A K G D A P C P	255
766	agaggggaagctggggccaggcagaggccaggagaatggagaggaggccaaaggaaactt	825
256	R G E A G C Q A E A R E I N G E E A K E L	275
826	ccagggaaacactggagtctaagaacacccaaaatgacttggaggcacattgttcaa	885
276	P G E T L E S K N T Q N D F E V H I V Q	295
886	gtggagaatgtgagatctaagatctaagatacaggtacccacagaagtctcgtgccag	945
296	V E N D E I	301
946	aacataagccctqaagtggcaggggaaatgtacgctggacaaggaccatctctgtgt	1005
1006	ccctgtctggcccaaggatcaggtatcaggtcttctatgcagctcaggayaccctaaagta	105
1066	agggcagattaccaataagaactgaatgaattcaaaaaaaaaaaaaaa	1113

SEQ I.D. NO.: 3 &amp; 4

## Human 19

1	ccgggcttacccagaga	caagaccctgatggctgcgg	gtttctggtaacgc	tttatgaa	60			
1	M A A V F L V T L Y E				11			
61	tactcgccg	ctttcacatcg	cggtgg	cttacatgc	tcatcg	gaccacgg	cct	120
12	Y S P L F Y I A V V F T C F I V T T G L							31
121	gtattggatgg	tttggatgttcc	aggtaattct	gagaaaattc	cagaaca	qaccca	s	180
32	V L G W F G W D V P V I S R N S E E T Q							51
181	ttcagcacaag	agtttcaaaa	agcataatg	agacaagtcaaga	atcc	tttgg	cttagas	240
52	F S T R V F K K Q M R Q V K N P F C L E							71
241	attcaactccatctt	cagttcaattaca	actggcataaac	tttgaca	acagat	ttggc	cct	300
72	I T N P S S A S I T T G I T L T T D C L							91
301	gaagatagcc	cttacatgctact	gggggtgc	agtgttcaaaa	attatcatg	aagctctg	360	
92	E D S L L T C Y W G C S V Q K L Y E A L							111
361	cagaagcatgtt	tattgttca	gataa	agcactccc	aaagcatt	aaagat	actctgtat	420
112	Q K H V Y C F R I S T P Q A L E D A L Y							131
421	agtgaatatctt	atccagga	acagtat	tttttattaaa	aggatag	caaaga	agaaatata	480
132	S E Y L Y Q E Q Y F I K K D S K E E I Y							151
481	tgccagg	taccaagagata	ctaaaatt	gaagactttgg	tacagtc	ccca	gat	540
152	C Q L P R D T K I E D F G T V P R S R Y							171
541	ccattggtagcg	ctattgac	cttagct	gtatgaggat	gaccgg	ggaaattt	tatcatatt	600
172	P L V A L L T L A D E D D R E I Y D I I							191
601	tccatgggtc	agtgttcatat	ttcgtat	aggacttataaa	actatctt	gc	aaatatty	660
192	S M V S V I H I P D R T Y K L S C F I L							211
661	tatcaatatttactt	ttggctcaagg	tcaattt	catgtat	ttaaycaac	tttcatgt	ct	720
212	Y Q Y L L A Q G Q F H D L K Q L F M S							231
721	gcaaataataatttactt	ccctccaacaattt	ccctt	caaga	aaaaaa	aaaaacaca	qacaca	780
232	A N N N F T P S N N S S S E E K N T D F							251
781	agtttgttgg	aaaagggtgg	actctct	gtaaagtgt	aaagtgt	gagccatcg	qaqadaac	840
252	S L L E K V G L S E S E V E P S E E N S							271
841	aaggactgtgtt	tttgc	cagaatgg	actgrga	actgg	actt	tatcat	900
272	K D C V V C O N G T V N W V L I I R E							291
901	acatgcctgtgt	gtatggctgt	gtgt	gaagtat	tttc	cagcgt	gc	960
292	T C L C D G V Y K Y F D G C D M V Q F							311
961	gttcaggaaat	ttttgcacttt	gcagtcaaaa	agagca	aaat	aaacaa	aaacaa	1020
312	V Q E S F A L C S Q K E Q D K D K P K T							331
1021	ctttgaagacatcg	taacactg	aaaagtac	actttt	ctact	aaat	aaat	1080
332	L .							15
1081	tcttggaaattc	atcataacat	ggaatct	acatgt	actqacc	atcaat	quaaat	1140
1141	aacttcata	tttgtat	ggacttgg	gtat	aaaaattt	atttt	tttgtat	1200
1201	atgaatactgg	aaatccat	ctgtgt	gtat	acaata	aaaaattt	ttgtat	1260
1261	aaaaaaaaaaaa	1273						

## SEQ I.D. NO.: 5 &amp; 6

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58	cacgaagccggagccccagacgcgcacaggccgcccagcagctccgggtttcagcagga	1
	M	1
2	tgtccccgtggctaattgttgcgtgcgcattgtgtccctttgggtcaag	61
2	S R W L M Q M L M P L L L P L G Q A	21
62	ctqcccccaaggatggagggttgcaaggctggaccctgaggcacaacagcagtcacaacca	121
22	A P K D G V A R L D P E A Q Q Q L T T N	41
122	accccttccagccaggccctgagcagctccgacgtctgcgggattatctcaaggactgg	181
42	P F Q P C P E Q L R R L R D Y L K G L E	61
182	agaagatggaagaggaccctgaacaaatgaaccgggagcaaqtcctgttatatcttcgg	241
62	K M E E D P E Q M N R E Q V L L Y L F A	81
242	ctcttcacgactttgaccagaacggacaactggacggccctggaaactctgtccatgtga	301
82	L H D F D C N G O L D G I E L L S M L T	101
302	cagcagctctggccctggagctgcacacttcccatcaacccggtgatccctggtagtag	361
102	A A L A P C A A H F P I N P V I L V V D	121
362	acatggtgcttgagactcaggacctggatggagacgggctcatgactccctgcagagctca	421
122	M V L E T Q D L D G D G L M T P A E L I	141
422	tcaactcccaggagaagcccccaagcgcgcagactccctccccagctctccaggagc	481
142	N F P G E A P K R A E S L P P A L Q E P	161
482	cacaacctgccggaaagtccggcttttagccaacagtcctcgatcaatcagaaaaccgc	541
162	Q P A G S Q P L L A N S P L Q S E T Q Q	181
542	aatccctggggactaaagaagaaattacaagtcaggttagaggccaaaaggccctggac	601
182	S L G T K E E I T S Q V E A K R A L E P	201
602	ctgaacaggaggctggacatcacatagagactaaagttagatgtcttaagccctgaagggg	661
202	E Q E A G H H I E T K V D A L S P E G E	221
662	aggctagggggtcaggcagactctgaaggagatccccaggccccggaggatgtgaga	721
222	A R G Q A E S E G D A P C P R E D A E R	241
722	gacagggtggagagcaaggacaacgaaaggaaaaagccaaagacactggccagcggaaacactag	781
242	Q V E S K D N E G E A K D L P A E T L E	261
782	agacccaaaaacactccaaatgtgggtgaggctcatagcatccaactggaaaacgacgaa	841
262	T Q N T P N V V E A H S I Q L E N D E :	281
842	tatgagctagacacacaggctcatgccccctcaggatctcagtgcagaacagaagcatcaa	901
	.	
902	gtgtgaatacagtggtctggagccacccatgcacatggtaatgggtccaaagctcgta	961
962	tcccaagaagcactgagagctccaggctagcctaggccacttagcaagtttgaagccaggc	1021
1022	tgggctacttcgtgaggccctgtttgaggagggaaaagaaaacgcttccacgttcc	1081
1082	tttctggccctcagtaggaagcaggacttttttcgcagctcagggagaccacaagctq	114!
1142	agaggcagatttcaggccccaaacaatgtggatgttgcataaaa	1201
1202	aaaaaaaa 1209	

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SEQ I.D. NO.: 7 &amp; 8

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-98	cctgggcgagtgaggacccacaaagttgtccgcctccggggcgcacataccagctcggttc	-39
-38	ccggccggccncrggtccgggcttagccaggagaccccgcgtggccgcgtgttccgtt	21
1	M A A V F L V	7
22	acgctctacgaataactcgccgctttctacatcgccgtggcttcacccgttcatcgcc	8:
8	T L Y E Y S P L F Y I A V V F T C F I A	27
82	accaccggctggatttagatggcttggctggacgttccagtaattctgagaaactca	141
28	T T G L V L G W L G W D V P V I L R N S	47
142	gaagaacccaggttcagcacaaagagcttcaagaaacaatgagacaagtgaaagaaatccg	201
48	E E T Q F S T R A F K K Q M R Q V K N P	67
202	tccggcttagagatactaactcatccgcagcttccctagcaacaggtgtaacccgttgcac	261
68	F C L E I T N S S A A S L A T G V T L T	87
262	acagactgcctggaaagacagtcgttacgtgtactgggggtgcgtgtaaaagctt	321
88	T D C L E D S R L T C Y W G C S V Q K L	107
322	tatgaagctttcagaagcacgttactgtctcaggataagcactccgcaagccctggaa	381
108	Y E A L Q K H V Y C F R I S T P Q A L E	127
382	gaggcgctgtacagtgactatctccaccgagaacagctttttttaaaagcacagcaa	441
128	E A L Y S D Y L H R E Q Y F I K K H S K	147
442	gaagaaatatattgccaatttaccaagcagtactgggttgaagactttggccgttgc	501
148	E E I Y C Q L P S S T G V E D F G P V ?	167
502	agatctcgctatccgttggtagcttgcgttgcgttatggatgacagggaaat	561
168	R S R Y P L V A L L T L A D E D D R E I	187
562	tatgacattattccatgggtctgtcattcatattccgtataagacttacaaacttcc	521
188	Y D I I S M V S V I H I P D K T Y K L P	207
622	tgcagaatattgtatcaaatattaaatccgttcaaggtaatttatgtatctaagcaa	681
208	C R I L Y Q Y L I L A Q G Q F Y D L K Q	227
682	cttttcatgtctgcaaataataggctactccccccagagaccaatctccscagacgg;	741
228	L F M S A N N S A T P S R D Q S P A D G	247
742	agtgtggagcacagcttgggagaaggcgccccctggctggctggatggaccgg;	801
248	S V E H S L L E K A G L A G A E V D P V	267
802	gaggagagcagcaaagactgtgtgggtgcagaacgggggtgtgaactgggtgttgc	861
268	E E S S K D <u>S V V C C N G G V N W V L L</u>	287
862	ccttgcggcacgcctgcgtgcacagctgtgtgttcaagcagtgtccca	921
288	<u>P C R H A C L C D S C V F K O C P M</u>	307
922	tgccgccagtttgtcaggaatcccttgcactgtgtgtcaaaaaaggccgacaaaggac	981
208	<u>C R Q F V Q E S F A L C G Q F E A D K D</u>	327
982	atactagaaaacttccatggatqacgggataaaagtcttctqctcag	104
328	I L E T S *	332
1042	cctcggtcccttagagtcaatccaaacagactgtgttgcgttccatggagagac	1101
1102	tgtgtgcactttcatactttgtacagtacatggatqacgggataaaagtcttctqctcag	1161
1162	tgcgaaaaaaaaaaaa	1176

5

**We Claim:**

1. An isolated and purified subchromosomal DNA molecule which encodes a mammalian CGR11 protein.
2. The isolated and purified subchromosomal DNA molecule of claim 1 which encodes a human CGR11 protein.
3. The DNA molecule of claim 2 which contains no intervening sequences.
4. The DNA molecule of claim 2 which comprises the sequence shown in SEQ ID NO:1.
5. The isolated and purified subchromosomal DNA molecule of claim 1 which encodes a rat CGR11 protein.
6. The DNA molecule of claim 5 which contains no intervening sequences.
7. The DNA molecule of claim 5 which comprises the sequence shown in SEQ ID NO:5.
8. An isolated and purified mammalian CGR11 protein.
9. The isolated and purified mammalian CGR11 protein of claim 8 consisting of the sequence of human CGR11 as shown in SEQ ID NO:2.
10. The isolated and purified mammalian CGR11 protein of claim 8 consisting of the sequence of rat CGR11 as shown in SEQ ID NO:6.
11. An antibody which specifically binds to a mammalian CGR11 protein.
12. The antibody which specifically binds to a mammalian CGR11 protein of claim 11, wherein the CGR11 is human and consists of the sequence shown in SEQ ID NO:2.
13. The antibody which specifically binds to a mammalian CGR11 protein of claim 11, wherein the CGR11 is rat and consists of the sequence shown in SEQ ID NO:6.

5           14. A method of suppressing growth of tumor cells, comprising the step  
of:

administering to said cells a mammalian CGR11 protein.

10          15. The method of claim 14 wherein the protein is human and consists  
of the sequence shown in SEQ ID NO:2.

15          16. The method of claim 14 wherein the protein is rat and consists of the  
sequence shown in SEQ ID NO:6.

20          17. A method of suppressing growth of tumor cells, comprising the step  
of:

25          administering to said cells a DNA molecule which causes said cells  
to express a mammalian CGR11 protein.

15          18. The method of claim 17 wherein the DNA molecule comprises the  
sequence shown in SEQ ID NO:1.

20          19. The method of claim 17 wherein the DNA molecule comprises the  
sequence shown in SEQ ID NO:5.

25          20. A method for diagnosing cancer, comprising the steps of:

assaying a tissue sample to measure the level of expression of a  
mammalian CGR11 protein or mRNA in the tissue;  
comparing that level with the level of expression found in a sample  
of a normal tissue, wherein a lower level of expression in the tissue sample than in  
the normal tissue sample is indicative of cancer.

30          21. The method of claim 20 wherein the step of assaying utilizes an  
antibody which is specifically reactive with a mammalian CGR11 protein.

22. The method of claim 21 wherein the mammalian CGR11 protein is  
human CGR11 as shown in SEQ ID NO:2.

30          23. The method of claim 21 wherein the mammalian CGR11 protein is  
rat CGR11 as shown in SEQ ID NO:6.

24. The method of claim 20 wherein the step of assaying utilizes a  
nucleic acid probe comprising at least 15 nucleotides complementary to a  
mammalian CGR11 mRNA.

5        25. The method of claim 24 wherein the probe has a sequence selected from SEQ ID NO:1.

10      26. The method of claim 24 wherein the probe has a sequence selected from SEQ ID NO:5.

15      27. A method for diagnosing cancer, comprising the steps of:

assaying a tissue to determine if DNA in said tissue contains a mutant form of a mammalian *CGR11* gene coding sequence, which differs from the wild-type form of the gene coding sequence.

20      28. The method of claim 27 wherein DNA of the tissue is compared to DNA of a normal tissue to determine whether the *CGR11* gene coding sequence is a mutant form.

25      29. The method of claim 27 wherein the wild-type gene coding sequence is shown in SEQ ID NO:1.

30      30. The method of claim 27 wherein the wild-type gene coding sequence is shown in SEQ ID NO:5.

35      31. An antisense *CGR11* construct comprising:

a. a transcriptional promoter;

b. a transcriptional terminator;

40      c. a DNA segment comprising one or more segments of a mammalian *CGR11* gene coding sequence, said gene coding sequence segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of *CGR11* RNA produced by mammalian cells.

45      32. The antisense *CGR11* construct of claim 31 wherein said transcriptional promoter is inducible.

50      33. The antisense *CGR11* construct of claim 31 wherein the mammalian *CGR11* gene coding sequence is a rat gene sequence shown in SEQ ID NO:5.

5           34. The antisense *CGR11* construct of claim 31 wherein the mammalian *CGR11* gene coding sequence is a human gene coding sequence shown in SEQ ID NO:1.

10           35. A *CGR11* antisense oligonucleotide comprising: at least ten nucleotides complementary to a mammalian *CGR11* mRNA.

15           36. The *CGR11* antisense oligonucleotide of claim 35 which comprises at least about twenty nucleotides complementary to the *CGR11* mRNA.

20           37. The *CGR11* antisense oligonucleotide of claim 35 which contains one or more modified nucleotide analogues.

25           38. The *CGR11* antisense oligonucleotide of claim 35 which is a circular molecule.

30           39. The *CGR11* antisense oligonucleotide of claim 35 which is complementary to the sequence of SEQ ID NO:1.

35           40. The *CGR11* antisense oligonucleotide of claim 35 which is complementary to the sequence of SEQ ID NO:5.

40           41. A method for promoting the proliferation of cells, comprising the step of:

45           administering a *CGR11* antisense oligonucleotide comprising at least ten nucleotides complementary to a mammalian *CGR11* mRNA to said cells to inhibit the expression of *CGR11*.

50           42. The method of claim 41 wherein the *CGR11* antisense oligonucleotide is complementary to rat *CGR11* as shown in SEQ ID NO:5.

55           43. The method of claim 41 wherein the *CGR11* antisense oligonucleotide is complementary to human *CGR11* as shown in SEQ ID NO:1.

60           44. A method for promoting the proliferation of cells, comprising the step of:

65           administering a *CGR11* triplex-forming oligonucleotide comprising at least ten nucleotides complementary to a mammalian *CGR11* gene to said cells to inhibit the expression of a mammalian *CGR11* gene.

5           45. The method of claim 44 wherein the oligonucleotide is complementary to rat *CGRII* as shown in SEQ ID NO:5.

10          46. The method of claim 44 wherein the oligonucleotide is complementary to human *CGRII* as shown in SEQ ID NO:1.

15          47. A method for promoting growth of cells, comprising the step of:  
                      administering to said cells to inhibit the expression of a mammalian *CGRII*, an antisense *CGRII* construct comprising:  
                      a. a transcriptional promoter;  
                      b. a transcriptional terminator;  
                      c. a DNA segment comprising one or more segments of the mammalian *CGRII* gene coding sequence, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of *CGRII* RNA produced by mammalian cells.

20          48. The method of claim 47 wherein said transcriptional promoter is inducible.

25          49. The method of claim 47 wherein the mammalian *CGRII* gene coding sequence is a rat gene sequence shown in SEQ ID NO:5.

50.         The method of claim 47 wherein the mammalian *CGRII* gene coding sequence is a human gene coding sequence shown in SEQ ID NO:1.

30          51. A method for assessing susceptibility to cancers, comprising the step of:

                     assaying a tissue selected from the group consisting of blood, mucosal smear, skin, cervical smear, chorionic villi, amniotic fluid, and a blastomere of a preimplantation embryo, to determine if DNA in said tissue contains a mutant mammalian *CGRII* gene coding sequence.

52.         The method of claim 51 wherein DNA is compared to a wild-type sequence of a human *CGRII* gene coding sequence.

5           53. The method of claim 52 wherein the wild-type sequence of the human gene coding sequence is shown in SEQ ID NO:1

10           54. An isolated and purified subchromosomal DNA molecule which encodes a mammalian CGR19 protein.

15           55. The isolated and purified subchromosomal DNA molecule of claim 54 which encodes human CGR19 protein as shown in SEQ ID NO:4.

20           56. The DNA molecule of claim 55 which contains no intervening sequences.

25           57. The DNA molecule of claim 55 which comprises the sequence shown in SEQ ID NO:3.

30           58. The isolated and purified subchromosomal DNA molecule of claim 54 which encodes rat CGR19 protein as shown in SEQ ID NO:8.

35           59. The DNA molecule of claim 58 which contains no intervening sequences.

40           60. The DNA molecule of claim 58 which comprises the sequence shown in SEQ ID NO:7.

45           61. An isolated and purified mammalian CGR19 protein.

50           62. The isolated and purified mammalian CGR19 protein of claim 61 consisting of the sequence of human CGR19 as shown in SEQ ID NO:4.

55           63. The isolated and purified mammalian CGR19 protein of claim 61 consisting of the sequence of rat CGR19 as shown in SEQ ID NO:8.

60           64. An antibody which specifically binds to a mammalian CGR19 protein.

65           65. The antibody of claim 64 which specifically binds to a mammalian CGR19 protein, wherein the CGR19 is human and consists of the sequence shown in SEQ ID NO:4.

70           66. The antibody of claim 64 which specifically binds to a mammalian CGR19 protein, wherein the CGR19 is rat and consists of the sequence shown in SEQ ID NO:8.

5           67. A method of suppressing growth of tumor cells, comprising the step  
of:

administering to said cells a mammalian CGR19 protein.

68. The method of claim 67 wherein the protein is human and consists  
of the sequence shown in SEQ ID NO:4.

10         69. The method of claim 67 wherein the protein is rat and consists of the  
sequence shown in SEQ ID NO:8.

70. A method of suppressing growth of tumor cells, comprising the step  
of:

15         administering to said cells a DNA molecule which causes said cells  
to express a mammalian CGR19 protein.

71. The method of claim 70 wherein the DNA molecule comprises the  
sequence shown in SEQ ID NO:3.

20         72. The method of claim 70 wherein the DNA molecule comprises the  
sequence shown in SEQ ID NO:7.

73. A method for diagnosing cancer, comprising the steps of:

assaying a test sample of a tissue to measure the level of expression  
of a mammalian CGR19 protein or mRNA;

comparing the level of expression of the test sample to the level of  
expression of a normal tissue, wherein a test sample having a lower level of  
expression than a normal tissue is indicative of cancer.

25         74. The method of claim 73 wherein the step of assaying utilizes an  
antibody which is specifically reactive with a mammalian CGR19 protein.

75. The method of claim 74 wherein the mammalian CGR11 protein is  
human CGR19.

30         76. The method of claim 75 wherein the humanCGR11 protein is as  
shown in SEQ ID NO:4.

77. The method of claim 73 wherein the step of assaying utilizes a  
nucleic acid probe comprising at least 15 nucleotides complementary to a  
mammalian CGR19 mRNA.

5        78.     The method of claim 77 wherein the probe has a sequence selected from contiguous nucleotides of SEQ ID NO:3.

10      79.     The method of claim 77 wherein the probe has a sequence selected from contiguous nucleotides of SEQ ID NO:7.

15      80.     A method for diagnosing cancer, comprising the steps of:

assaying a test tissue to determine if DNA in said tissue contains a mutant form of a mammalian *CGR19* gene coding sequence, wherein a mutant form differs from the wild-type form of the gene coding sequence.

20      81.     The method of claim 80 wherein DNA of the tissue is compared to DNA of a normal tissue to determine whether the *CGR19* gene coding sequence is a mutant form.

25      82.     The method of claim 80 wherein the wild-type gene coding sequence is shown in SEQ ID NO:3.

30      83.     The method of claim 80 wherein the wild-type gene coding sequence is shown in SEQ ID NO:7.

35      84.     An antisense *CGR19* construct comprising:

a. a transcriptional promoter;

b. a transcriptional terminator;

40      c. a DNA segment comprising one or more segments of a mammalian *CGR19* gene coding sequence, said gene coding sequence segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of *CGR19* RNA produced by mammalian cells.

45      85.     The antisense *CGR19* construct of claim 84 wherein said transcriptional promoter is inducible.

50      86.     The antisense *CGR19* construct of claim 84 wherein the mammalian *CGR19* gene coding sequence is a rat gene sequence shown in SEQ ID NO:7.

5           87. The antisense *CGR19* construct of claim 84 wherein the mammalian *CGR19* gene coding sequence is a human gene coding sequence shown in SEQ ID NO:3.

10          88. A *CGR19* antisense oligonucleotide comprising: at least ten nucleotides complementary to a mammalian *CGR19* mRNA.

15          89. The *CGR19* antisense oligonucleotide of claim 88 which comprises at least about twenty nucleotides complementary to the *CGR19* mRNA.

20          90. The *CGR19* antisense oligonucleotide of claim 88 which contains one or more modified nucleotide analogues.

25          91. The *CGR19* antisense oligonucleotide of claim 88 which is a circular molecule.

30          92. The *CGR19* antisense oligonucleotide of claim 88 which is complementary to the sequence of SEQ ID NO:3.

35          93. The *CGR19* antisense oligonucleotide of claim 88 which is complementary to the sequence of SEQ ID NO:7.

40          94. A method for promoting the proliferation of cells, comprising the step of:

45              administering a *CGR19* antisense oligonucleotide comprising at least ten nucleotides complementary to a mammalian *CGR19* mRNA to said cells to inhibit the expression of *CGR19*.

50          95. The method of claim 94 wherein the *CGR19* antisense oligonucleotide is complementary to rat *CGR19* as shown in SEQ ID NO:7.

55          96. The method of claim 94 wherein the *CGR19* antisense oligonucleotide is complementary to human *CGR19* as shown in SEQ ID NO:3.

60          97. A method for promoting the proliferation of cells, comprising the step of:

65              administering a *CGR19* triplex-forming oligonucleotide comprising at least ten nucleotides complementary to a mammalian *CGR19* gene to said cells to inhibit the expression of a mammalian *CGR19* gene.

5           98. The method of claim 97 wherein the oligonucleotide is complementary to rat *CGR19* as shown in SEQ ID NO:7.

10          99. The method of claim 97 wherein the oligonucleotide is complementary to human *CGR19* as shown in SEQ ID NO:3.

15          100. A method for promoting growth of cells, comprising the step of:  
                       administering to said cells to inhibit the expression of a  
                       mammalian *CGR19*, an antisense *CGR19* construct comprising:  
                       a. a transcriptional promoter;  
                       b. a transcriptional terminator;  
                       c. a DNA segment comprising one or more segments of the  
                       mammalian *CGR19* gene coding sequence, said gene segment located between said  
                       promoter and said terminator, said DNA segment being inverted with respect to said  
                       promoter and said terminator, whereby RNA produced by transcription of the DNA  
                       segment is complementary to a corresponding segment of *CGR19* RNA produced  
                       by mammalian cells.

20          101. The method of claim 100 wherein said transcriptional promoter is  
                       inducible.

102. The method of claim 100 wherein the mammalian *CGR19* gene  
                       coding sequence is a rat gene sequence shown in SEQ ID NO:7.

25          103. The method of claim 100 wherein the mammalian *CGR19* gene  
                       coding sequence is a human gene coding sequence shown in SEQ ID NO:3.

104. A method for assessing susceptibility to cancers, comprising the step  
                       of:

30          assaying a tissue selected from the group consisting of blood,  
                       chorionic villi, amniotic fluid, and a blastomere of a preimplantation embryo, to  
                       determine if DNA in said tissue contains a mutant mammalian *CGR19* gene coding  
                       sequence.

105. The method of claim 104 wherein wild-type sequence of the  
                       mammalian *CGR19* gene coding sequence is shown in SEQ ID NO:3.

5           106. The method of claim 104 wherein wild-type sequence of the mammalian *CGR19* gene coding sequence is shown in SEQ ID NO:7.

10           107. A method of suppressing growth of tumor cells, comprising the step of:

               administering to said cells a mammalian *SM20* or *mEH* protein.

15           108. A method of suppressing growth of tumor cells, comprising the step of:

               administering to said cells a DNA molecule which causes said cells to express a mammalian *SM20* or *mEH* protein.

15           109. A method for diagnosing cancer, comprising the steps of:

               assaying a tissue to determine if the tissue expresses less of a mammalian *SM20* or *mEH* protein or mRNA than a normal tissue.

20           110. A method for diagnosing cancer, comprising the steps of:

               assaying a tissue to determine if DNA in said tissue contains a mutant form of a mammalian *SM20* or *mEH* gene coding sequence, which mutant form differs from the wild-type form of the gene coding sequence.

25           111. A method for promoting the proliferation of cells, comprising the step of:

               administering a *SM20* or *mEH* antisense oligonucleotide comprising at least ten nucleotides complementary to a mammalian *SM20* or *mEH* mRNA to said cells to inhibit the expression of *SM20* or *mEH*.

30           112. A method for promoting the proliferation of cells, comprising the step of:

               administering a *SM20* or *mEH* triplex-forming oligonucleotide comprising at least ten nucleotides complementary to a mammalian *SM20* or *mEH* gene to said cells to inhibit the expression of a mammalian *SM20* or *mEH* gene.

35           113. A method for promoting growth of cells, comprising the step of:

               administering to said cells to inhibit the expression of a mammalian *SM20* or *mEH*, an antisense *SM20* or *mEH* construct comprising:

               a. a transcriptional promoter;

b. a transcriptional terminator;  
c. a DNA segment comprising one or more segments of the mammalian *SM20* or *mEH* gene coding sequence, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of *SM20* or *mEH* RNA produced by mammalian cells.

114. A method for assessing susceptibility to cancers, comprising the step  
of:

assaying a tissue selected from the group consisting of blood, chorionic villi, amniotic fluid, and a blastomere of a preimplantation embryo, to determine if DNA in said tissue contains a mutant mammalian *SM20* or *mEH* gene coding sequence.

115. A pharmaceutical composition comprising an effective amount of a mammalian protein selected from the group consisting of *SM20*, *mEH*, *CGR11*, and *CGR19*, wherein the pharmaceutical composition is suitable for intranasal or parenteral administration.

116. A method of preparing an expression vector capable of producing a mammalian protein selected from the group consisting of *SM20*, *mEH*, *CGR11*, and *CGR19*, the method comprising:

ligating in an operative position and orientation, a DNA encoding the protein to a vector such that the DNA encoding the protein can be expressed in a host cell.

117. An expression vector capable in a host cell of producing a mammalian protein selected from the group consisting of *SM20*, *mEH*, *CGR11*, and *CGR19*, the vector comprising:

a DNA segment encoding the selected protein; and  
a vector, wherein the DNA segment is linked in an operative  
position and orientation, such that the DNA encoding the protein can be expressed  
in a host cell.

5           118. A host cell comprising an expression vector capable of directing expression of a mammalian protein selected from the group consisting of *SM20*, *mEH*, *CGR11*, and *CGR19*.

10          119. The method of claim 14, 67, or 107, wherein the cells are in a mammalian body and a tumor suppressing amount of the protein is administered.

110         120. An *SM20* or *mEH* antisense oligonucleotide comprising at least ten nucleotides complementary to a mammalian *SM20* or *mEH* mRNA..

115         121. An *SM20* or *mEH* triplex-forming oligonucleotide comprising at least ten nucleotides complementary to a mammalian *SM20* or *mEH* aid cells to inhibit the expression of a mammalian *SM20* or *mEH* gene.

120         122. An antisense *SM20* or *mEH* construct comprising:

- a. a transcriptional promoter;
- b. a transcriptional terminator;

125         c. a DNA segment comprising one or more segments of the mammalian *SM20* or *mEH* gene coding sequence, said gene segment located between said promoter and said terminator, said DNA segment being inverted with respect to said promoter and said terminator, whereby RNA produced by transcription of the DNA segment is complementary to a corresponding segment of *SM20* or *mEH* RNA produced by mammalian cells.

130         123. An isolated DNA sequence encoding a mammalian CGR protein obtained by a method comprising the steps of:

135           annealing at least one set of mixed oligonucleotides to a mammalian cDNA library, each member of said set of mixed oligonucleotides encoding a sequence of at least six contiguous amino acids of the amino acid sequence shown in SEQ ID NO:2, 4, 6, or 8;

140           isolating a mammalian cDNA which (1) anneals to at least one member of the set of mixed oligonucleotides, (2) contains a complete open reading frame of about 275 to about 335 codons and (3) encodes a mammalian CGR protein, wherein a CGR RNA is upregulated in the presence of wild-type p53.

5           124. The mammalian CGR DNA sequence of claim 1 wherein at least two sets of mixed oligonucleotides are annealed.

Table 1 *Inhibition of colony formation by Cell Growth Regulatory Genes*

Clone	Colony number <sup>a</sup> (% of control <sup>b</sup> )		
	SW480	SKOV3 IP1	T98G
hWAF1	29.0 ± 3.4	34.5 ± 6.8	20.6 ± 8.6
hp53	0.2 ± 0.2	5.1 ± 1.4	5.7 ± 2.3
hp53(as)	92.7 ± 37.0	62.4 ± 20.1	151.5 ± 29.0
rCGR11	18.2 ± 8.8	31.5 ± 9.3	25.8 ± 11.3
rSM20	4.4 ± 3.6	16.4 ± 8.3	11.4 ± 3.2
rmEH	73.6 ± 6.1	21.9 ± 7.3	5.2 ± 3.2
rCGR19	74.8 ± 7.7	38.3 ± 17.5	13.3 ± 7.6
hCGR11	N.D.	8.3 ± 0.1	57.6 ± 11.7
hCGR11ΔEF	N.D.	110.0 ± 5.0	98.8 ± 2.6

<sup>a</sup> Experiments were performed in duplicate<sup>b</sup> pCEP4 vector alone

N.D., not determined

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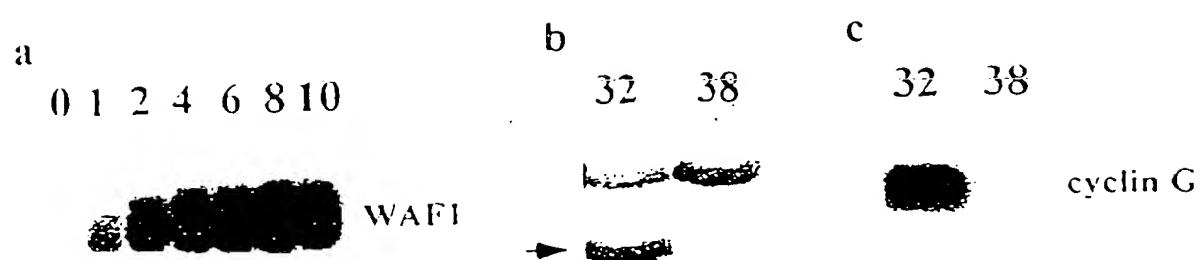


FIGURE 1

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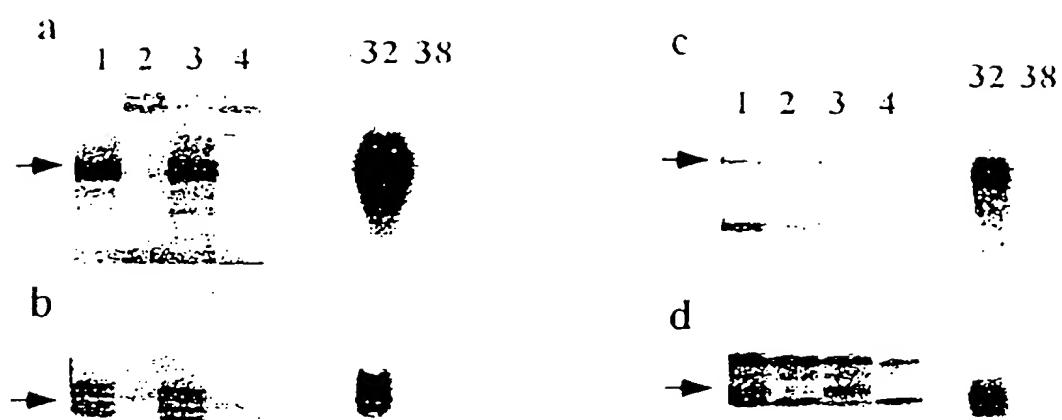
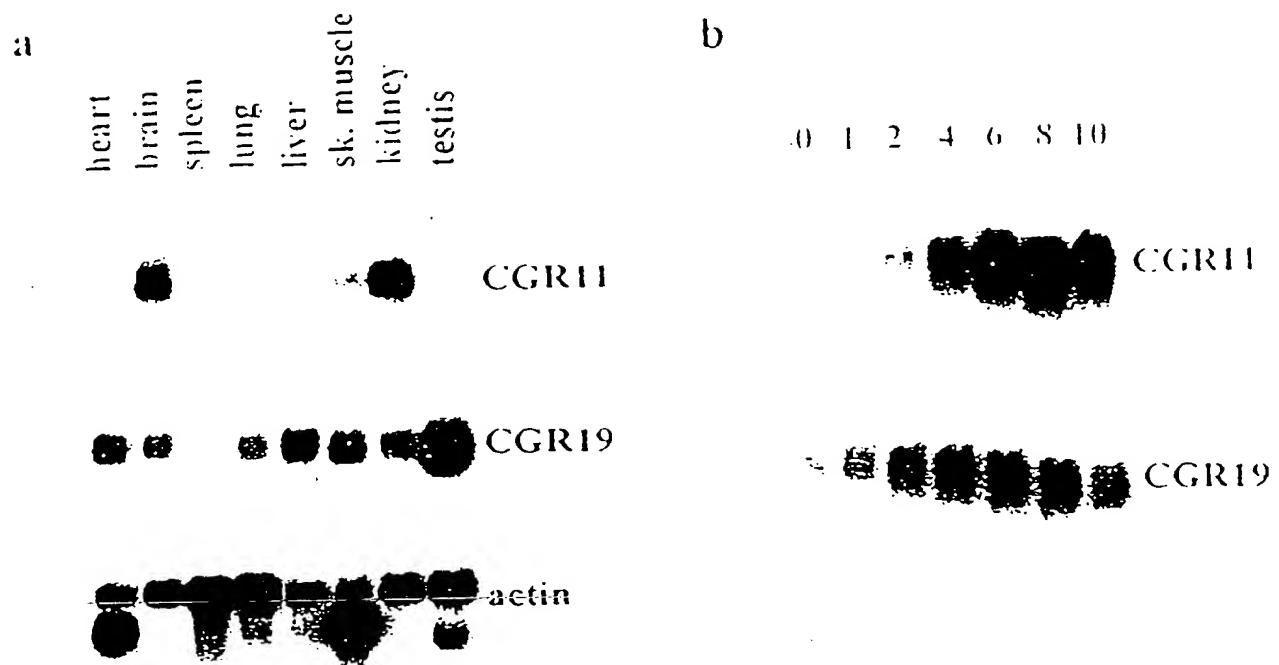


FIGURE 2

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**FIGURE 3**

a

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b

Dx ( DNS ) x ( DENSTG ) ( DNOGHRK ) x ( LIV11C ) ( DENSTAG ) x x ( DE ) ( LIV11EW )

I. DY--D--Q---S-----G---Q---I,-----D-----GL--R---I,  
F N

II. DL--N--G---D-----G---L---M-----T-----PA--E----L  
D

C

```

human (1) MAAVFLVTLYEYSPLFYIAVVFCTFIVTTGLVLGWFWDVPVILRNSEETQFSTRVFKKOM
rat      -----A-----L-----A-----

(62) RQVKNPFGLEITNPSSASITTCITLTDCLEDSSLTCYWGCSVQKLYEALQKHVYCFRIST
-----S-A--LA--V-----R-----

(123) PQALEDALYSEYLYQEQQYFIKKDSKEEITYCQLPRDTKIEDFGTVPRSRYPLVALLTADED
-----E---D--HR-----H-----SS-GV-----P-----

(184) DREIYDIISMVSVIHIPDRTYKLSMRIYQYLLAQQQFHDLKQLFMSANNNFTPSNNSSS
-----K---P-----I-----Y-----SA---RDQ-P-----

(245) EEKNTDRSLLEKVGLSESEVEPSEENSKDCVVCQNGTVNWLLPCRHTCLCDGCVKYFOOC
ADGSVEH----A---AGA--D-V--S-----G-----A---S--C--K---

(306) PMCRQFVQESFALCSQKEQDKDKPKTL (332)
-----G---A---I/I-E-S

```

d

CxxC-x(9-27)-Cx(1-3)Hxx-CxxCx(3-47)CxxC      Consensus  
 |    |            |    |    |    |    |  
 CVVC--x(11)--Cx(1 )HTCLCDGCx( 6 )CPMC      CGR19

## FIGURE 4



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: <b>C12N 15/12, C07K 14/47, 16/18, A61K 38/17, G01N 33/68, C12Q 1/68, C12N 15/11, A61K 31/70 // C12N 15/55, 9/14</b>		A3	(11) International Publication Number: <b>WO 97/45542</b> (43) International Publication Date: <b>4 December 1997 (04.12.97)</b>
<p>(21) International Application Number: <b>PCT/US97/09584</b></p> <p>(22) International Filing Date: <b>29 May 1997 (29.05.97)</b></p> <p>(30) Priority Data: 60/018,557                    29 May 1996 (29.05.96)                    US</p> <p>(71) Applicant: PHARMAGENICS, INC. [US/US]; 4 Pearl Court, Allendale, NJ 07401 (US).</p> <p>(72) Inventors: BEAUDRY, Gary, A.; 108 Inwood Avenue, Upper Montclair, NJ 07043 (US). BERTELSEN, Arthur, H.; 215 Manor Road, Ridgewood, NJ 07450 (US). GALELLA, Elizabeth, A.; 572 Mountain Avenue, Washington Township, NJ 07645 (US). MADDEN, Stephen, I.; 12-C Station Road, Cranbury, NJ 08512 (US).</p> <p>(74) Agents: KAGAN, Sarah, A. et al.; Banner &amp; Witcoff, Ltd., Suite 1100, 1001 G Street, N.W., Washington, DC 20001 (US).</p>			
<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.</i>  <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> </p> <p>(88) Date of publication of the international search report: <b>7 May 1998 (07.05.98)</b></p>			
<p><b>(54) Title:</b> CELL GROWTH REGULATORY GENES</p> <p><b>(57) Abstract</b></p> <p>Transcriptionally regulated growth-response genes play a pivotal role in the determination of a cell's fate. <i>p53</i> is known to transcriptionally regulate genes important in regulating cell growth potential. Using differential RT-PCR analysis of rat embryo fibroblast cells containing a temperature-sensitive <i>p53</i> allele, we were able to isolate several transcripts upregulated specifically in cells harboring the wild-type <i>p53</i> protein. Two of these genes, <i>SM20</i> and microsomal epoxide hydrolase (<i>mEH</i>), are previously described genes. Two previously uncharacterized cDNA's, cell growth regulatory (<i>CGR</i>) genes <i>CGR11</i> and <i>CGR19</i>, were isolated. The predicted amino acid sequences of these novel proteins contain known motifs: EF-hand domains (<i>CGR11</i>) and a ring-finger domain (<i>CGR19</i>), are suggestive of function. <i>CGR11</i> and <i>CGR19</i> appear to be primary response genes expressed at 0.05 % and 0.01 % of the total mRNA in wild-type <i>p53</i> cells. Both <i>CGR11</i> and <i>CGR19</i> as well as <i>SM20</i> and <i>mEH</i>, are able to inhibit growth of several cell lines.</p>			
<p><b>a</b></p> <p>human (1) MQLTHTVLTLPLLPTGQAARKDGVTGPDPSEVQHLLPMPFQICCGEQLLQSLSYKLKGIAHT rat (1) -----I-----A-L-P-A-Q-TT-----P---RR-RD----IKA</p> <p>(62) EVOLENLSEEVVLVLFALHOHDQSGOLDGELII.SHLTAALATCAGAANSRTTNAVILIVVH (55) -QH-----S-----N-----I-----HIF-----V-----I</p> <p>(123) I.MTQDLNGGCLMTPAELINFPQVALRHMVEPGFPLAPSQEPQCAVGROSLLAKSTLKGDIQH (115) -----D-----E-PKR***S-P-Al,---PA-S-P---N---O---I---</p> <p>(104) ATGPREAKGCOVARRESLDPVQFCGGCAEAATCIVCPGRCARCGOAEEAKGIAAATPQHGA (173) SI-TK---ITs---K-A-E-E-----I-----GHN1-T-V---I-----</p> <p>(245) QAEAKCDAPGPPGEAUQCGAEAKENGEAK-EI-INSETLESKNTNDFEVIIIVQVHNID (131) (216) ---SE-----ED-ER-V-SKD-EQ---D---A---T---P-VV-A-SI-----I-----</p> <p><b>b</b></p> <p>Dx(DNS)x(DENSTG)(DNQCHRK)x(LIVNC)(DENQSTAG)xx(DEI)(LIVHCFH)</p> <p>I. D-Y-U-O---S-----C---O---I,-----D-----CI,---E-----I, F-----N-----</p> <p>II. Dl---N---C---D-----C---L-----T-----PA---E-----L D</p> <p><b>c</b></p> <p>human (1) MAAVFLVLTLYEYSPFLYIAVVFPCFIVTTGLVLGWPQWDVPVILRNSEETOFSTRVFKKM rat -----A-----L-----A-----A-----</p> <p>(62) RQVKNPFGLEITPSSAATTCGTLLTDCLEDLSLTCTYNGCSVQKLYEALQKHYCVRIST -----S-A---LA---V-----R-----</p> <p>(123) PQALEDALYSELYQEOYFIKKDSKEEYICOLPRDTKIEDPGTVPRSRYPLVALI.TLAEDU -----E---D-MR-----H-----SS-CV-----P-----</p> <p>(184) DREIYDISHVSVIHPDTYKLSCLRLYQYLALLAQOFHDLKQLMFSANNNFTPSNNSSS -----K---P-----I-----Y-----SA---RDO-P-----</p> <p>(245) EKMTDRSLLCKVQLESEISVPSKEMSKDCVVCONGTVVNVVLLPCHRHTCLGCCCVKYFOOT AIGSVHE-----A---ACA---D-V-S-----C-----A---S---C---K-----</p> <p>(306) FMCROFVQESPALCSQEKODDKPKTL (332) -----G---A---ILE-S-----</p> <p><b>d</b></p> <p>CxxC---(9-27)-Cx(1-3)Kxx-CxxCx(1-67)CxxC      Consensus                   CVVC---x(11)--Cx( 1 ) IHTCLCDQCa( 6 ) ICPMC      CGR19</p>			

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09584

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C12N15/12 C07K14/47 C07K16/18 A61K38/17 G01N33/68  
 C12Q1/68 C12N15/11 A61K31/70 //C12N15/55,  
 C12N9/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 13375 A (UNIV JOHNS HOPKINS) 18 May 1995  see the whole document --- OKAMOTO K. AND BEACH D.: "Cyclin G is a transcriptional target of the p53 tumor suppressor protein." EMBO JOURNAL, vol. 13, no. 19, 1994, pages 4816-4822, XP002046024 see the whole document --- - / --	1-53, 115-119, 123,124
Y		1-53, 115-119, 123,124

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

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Date of the actual completion of the international search

7 November 1997

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

Internatinal Application No

PCT/US 97/09584

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MICHALOVITZ D. ET AL.: "Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53." CELL, vol. 62, 1990, pages 671-680, XP002046026 see the whole document	1-13, 31-40, 116-118, 123,124
A	---	14-30, 41-53, 115,119
Y	BAUER D. ET AL.: "Identification of differentially expressed mRNA species by an improved display technique." NUCLEIC ACIDS RESEARCH, vol. 21, no. 18, 1993, pages 4272-4280, XP000394394 see the whole document	1-13, 31-40, 116-118, 123,124
A	WO 96 01907 A (SQUIBB BRISTOL MYERS CO) 25 January 1996 see the whole document	1-53, 115-119, 123,124
A	EL-DEIRY W.S. ET AL.: "WAF1, a potential mediator of p53 tumor suppression." CELL, vol. 75, 1993, pages 817-825, XP002046022 see the whole document	1-53, 115-119, 123,124
A	BUCKBINDER L. ET AL.: "Gene regulation by temperature-sensitive p53 mutants: Identification of p53 response genes." PROC. NATL. ACAD. SCI. USA, vol. 91, 1994, pages 10640-10644, XP002046023 see the whole document, especially page 10640, from left column, last line to right column second line and page 10644, left column, last paragraph	1-53, 115-119, 123,124
A	EMBL database entry HS52516; accession number T33525; 9. September 1995; Adams M.D. et al.: 'Initial assessment of human gene diversity and expression patterns based upon 52 million basepairs of cDNA sequence.' XP002046045 see abstract	1-10, 31-40
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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09584

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MADDEN S.L. ET AL.: "Induction of cell growth regulatory genes by p53." CANCER RESEARCH, vol. 56, 1 December 1996, pages 5384-5390, XP002046025 see the whole document -----	1-53, 115-119, 123,124

# INTERNATIONAL SEARCH REPORT

i national application No.

PCT/US 97/09584

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 14-19, 41-50 and 119, as far as they are concerning an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-53 and 124 (complete), 115-119 and 123 (partially)

Remark on Protest

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/09584

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-53 and 124 (complete),  
115-119 and 123 (partially)

DNA molecule encoding CGR11, expression vector and host cell containing said DNA molecule; mammalian CGR11; antibody against mammalian CGR11; antisense molecules targeted against CGR11; method of suppressing growth of tumor cells by administering CGR11 or the DNA molecule that encodes CGR11; method for diagnosing cancer by measuring expression level of CGR11 or the corresponding mRNA or by identifying a mutation in the CGR11 gene; method for promoting cell proliferation by administering antisense molecules targeted against CGR11; method for assessing the susceptibility to cancer by identifying a mutation in the CGR11 gene; pharmaceutical composition comprising CGR11;

2. Claims: 54-106 (complete), 115-119 and 123 (partially)

DNA molecule encoding CGR19, expression vector and host cell containing said DNA molecule; mammalian CGR19; antibody against mammalian CGR19; antisense molecules targeted against CGR19; method of suppressing growth of tumor cells by administering CGR19 or the DNA molecule that encodes CGR19; method for diagnosing cancer by measuring expression level of CGR19 or the corresponding mRNA or by identifying a mutation in the CGR19 gene; method for promoting cell proliferation by administering antisense molecules targeted against CGR19; method for assessing the susceptibility to cancer by identifying a mutation in the CGR19 gene; pharmaceutical composition comprising CGR19;

3. Claims: 107-122 (partially)

expression vector and host cell containing DNA molecule encoding mEH and host cell containing said expression vector; antisense molecules targeted against mEH; method of suppressing growth of tumor cells by administering mEH or the DNA molecule that encodes mEH; method for diagnosing cancer by measuring expression level of mEH or the corresponding mRNA or by identifying a mutation in the mEH gene; method for promoting cell proliferation by administering antisense molecules targeted against mEH; method for assessing the susceptibility to cancer by identifying a mutation in the mEH gene; pharmaceutical composition comprising mEH

4. Claims: 107-122 (partially)

expression vector and host cell containing DNA molecule encoding SM20 and host cell containing said expression vector; antisense molecules targeted against SM20; method of suppressing growth of tumor cells by administering SM20 or

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 97/09584

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the DNA molecule that encodes SM20; method for diagnosing cancer by measuring expression level of SM20 or the corresponding mRNA or by identifying a mutation in the SM20 gene; method for promoting cell proliferation by administering antisense molecules targeted against SM20; method for assessing the susceptibility to cancer by identifying a mutation in the SM20 gene; pharmaceutical composition comprising SM20

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Initial Application No

PCT/US 97/09584

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9513375 A	18-05-95	NONE	
WO 9601907 A	25-01-96	US 5667987 A EP 0804609 A	16-09-97 05-11-97